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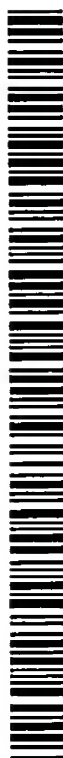
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(54) Title: **IMMUNOLOGIC METHOD FOR THE PREVENTION OF DENTAL CARIES**

(57) Abstract: Dental caries in man may be prevented or treated by oral ingestion of human or humanized mouse monoclonal IgG and IgM antibodies that bind to surface antigens of cariogenic organisms, such as *S. mutans*. The genetically immune system when they bind to cariogenic organisms, resulting in their destruction. In a preferred embodiment, monoclonal antibodies to cariogenic organisms are produced by edible plants, including fruits and vegetables, transformed by DNA sequences that code on expression for the desired antibodies. The antibodies are applied by eating the plants.

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IMMUNOLOGIC METHOD FOR THE PREVENTION OF DENTAL CARIES

Background of the Invention

5 This application relates to an immunologic methodology for the treatment and prevention of dental caries. This invention has special application to patients who are without the ability or motivation to apply established principles of self care, such as very young children, the infirm and poorly educated populations.

10 Dental caries (tooth decay) and periodontal disease are probably the most common chronic diseases in the world. The occurrence of cavities in teeth is the result of bacterial infection. Hence the occurrence of dental caries is properly viewed as an infectious microbiological disease that results in localized destruction of the calcified tissues of the teeth.

15 *Streptococcus mutans* is believed to be the principal cause of tooth decay in man. When *S. mutans* occurs in large numbers in dental plaque, and metabolizes complex sugars, the resulting organic acids cause demineralization of the tooth surface. The result is carious lesions, commonly known as cavities. Other organisms, such as *Lactobacilli* and *Actinomyces* are also believed to be involved in the progression and formation of carious lesions. Those organizations that cause tooth decay are referred to herein as "cariogenic organisms."

20 Removal of the damaged portion of a tooth and restoration by filling can, at least temporarily, halt the damage caused by oral infection with cariogenic organisms. However, the "drill and fill" approach does not eliminate the causative bacterial agent. Proper oral hygiene can control the accumulation
25 of dental plaque, where cariogenic organisms grow and attack the tooth surfaces.

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However, dental self-care has its limits, particularly in populations that are unable to care for themselves, or where there is a lack of knowledge of proper methods of self care. Administration of fluoride ion has been shown to decrease, but not eliminate the incidence of dental caries.

In view of the overwhelming evidence of the involvement of cariogenic organisms in the pathogenesis of dental caries, it is not surprising that there have been a number of different attempts to ameliorate the condition using traditional methods of anti-microbial therapy. The disadvantage of antimicrobial agents is that they are not selective for cariogenic organisms. Administration of non-specific bacteriocidal agents disturbs the balance of organisms that normally inhabit the oral cavity, with consequences that cannot be predicted, but may include creation of an environment that provides opportunities for pathogenic organisms. In addition, long term use of antimicrobial agents is known to select for organisms that are resistant to them. Hence long term and population-wide use of antimicrobial agents to prevent tooth decay is not practical.

Vaccination of humans to elicit an active immune response to *S. mutans*, or other cariogenic organisms, is also not a practical solution at this time. One drawback of this approach is that vaccination elicits production of predominantly IgG and IgM antibodies, but they are not secreted into saliva. The majority of antibodies present in saliva are of the IgA isotype, which can bind to, but cannot activate lymphocytes or complement components to kill bacteria. Accordingly, vaccination is not believed likely to be capable of producing antibodies that can trigger the immune system to kill cariogenic organisms in the mouth. There is no known method for selectively increasing the titer of vaccination induced antibodies of the IgG or IgM isotypes in the oral cavity.

There have been a number of reported attempts to passively immunize patients to *S. mutans* using monoclonal IgA antibodies raised in mice to prevent tooth decay in animals and in man. Because IgA is a multivalent antibody, a single molecule of IgA can bind to several different antigenic sites, resulting in clumping of bacteria. However, binding of IgA to bacterial surface antigens does not

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kill the bacteria. Rather, clumping is believed to hinder the ability of bacteria to bind to tooth surfaces. Another drawback of this approach is that repeated administration of mouse (i.e., heterologous) antibodies to humans has the potential to evoke an immune response to the antibodies.

5 Unlike IgA antibodies, antibodies of the IgG and IgM classes have bacteriocidal effects. Binding of IgM or IgG antibodies to antigens present on the surface of cariogenic organisms may result in the destruction of the bacterial cells by two separate mechanisms: complement mediated cell lysis and antibody-dependent cell-mediated cytotoxicity. In either case, antibodies that selectively
10 bind to certain microbial organisms target just those cells for destruction by the immune system. Both complement mediated cell lysis and antibody-dependent cell mediated cytotoxicity are part of the humoral immune response that is mediated by antibodies of the IgG and IgM classes.

In order to elicit the desired cytotoxic effect of antibody binding, monoclonal antibodies to cariogenic organisms must be recognized by the human immune system. There are a number of different technologies by which
15 antibodies that will trigger a response from a heterologous mammalian immune system can be produced. One example is a nucleic acid construct that codes on expression for a human antibody modified to incorporate sequences encoding the antigen specific binding domain from heterologous organisms.

Production and administration of such genetically engineered monoclonal antibodies to treat dental caries in man poses issues susceptible to a particularly innovative solution. Prior art methods for production of monoclonal
20 antibodies involve growing hybridomas in culture media, followed by extraction and purification of the desired antibody. These steps are significantly simplified in a preferred embodiment of the invention by expressing the antibodies in edible plants or animals (eukaryotes). The antibodies are administered upon oral ingestion of d plant or animal products, such as fruits, vegetables or milk wherein
25 the antibodies are not denatured. This mode of administration has the potential for obviating compliance issues in ameliorating tooth decay.

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Summary of the Preferred Embodiments

5 Dental caries may be prevented or treated by oral ingestion of
human or humanized mouse monoclonal IgG and IgM antibodies that to bind
surface antigens of cariogenic organisms, such as *S. mutans*. The genetically
engineered monoclonal antibodies engage the effector apparatus of the human
immune system when they bind to cariogenic organisms, resulting in their
10 destruction. In a preferred embodiment, monoclonal antibodies to cariogenic
organisms are produced by edible plants, including fruits and vegetables,
transformed by DNA sequences that code on expression for the desired
antibodies. The antibodies are applied by eating the plants.

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Detailed Description of the Preferred Embodiments1. Preparation of Monoclonal Antibodies

5 The monoclonal antibody technique permits preparation of a source of antibodies with extraordinary specificity. Monoclonal antibodies that bind to specific molecular structures can be produced using what are today considered standard techniques.

10 The monoclonal antibodies that may be used in this invention are those that are directed to surface antigens of cariogenic organisms. Surface antigens are substances that are displayed on the surface of cells. Such antigens are accessible to antibodies present in body fluids. In the context of the present invention, surface antigens of cariogenic organisms are present on the surface of organisms that cause dental caries. While the role of bacterial activity in the genesis of carious lesions is well defined, establishing a cause and effect relationship between a particular organism and the occurrence of dental caries has not been completely successful. To date, only *S. mutans* has been definitively associated with dental caries. However, species of the *Lactobacilli* and *Actinomyces* are also believed to be involved, particularly with the active progression of carious lesions. Any organism that can produce a carious lesion is a potential target for the monoclonal antibodies prepared and used in accordance with this invention.

20 A further requirement of the monoclonal antibodies that may be used in the practice of the present invention is that they are selective for cariogenic organisms. Monoclonal antibodies directed to antigens present on cariogenic as well as non-cariogenic organisms may produce non-specific alterations in the makeup of the flora within the oral cavity. The consequences of such changes are not understood.

25 Accordingly, the preferred monoclonal antibodies are directed to surface antigens of cariogenic organisms. That is to say, the preferred monoclonal antibodies bind specifically to organisms that cause dental caries.

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It should be clearly understood that the scope of the present invention is not limited to the prevention of tooth decay in man. Monoclonal antibodies in accordance with the present invention can be genetically engineered to engage the effector response of the immune system of other mammals, such as those that are domesticated as pets.

Monoclonal antibodies are prepared by immunizing mice or other mammalian hosts with cell wall material isolated from cariogenic organisms. In a preferred embodiment, the cariogenic organisms are type c *S. mutans* (ATCC25175). The immunogenicity of molecules present in cell walls may be enhanced by a variety of techniques known in the art. In a preferred embodiment, immunogenicity of such molecules is enhanced by denaturation of the isolated cell material with formalin. Other techniques for modifying cell wall proteins to enhance immunogenicity are within the scope of this invention. Typically, hosts receive one or more subsequent injections of isolated bacterial cell fragments to increase the titer of antibodies prior to sacrifice and cloning.

Spleen cells from hosts are harvested and cloned by limiting dilution using techniques that have become standard since the pioneering work of Kohler and Milstein. In a preferred embodiment, surviving hybridomas are screened for antibody directed to cariogenic organisms by ELISA assay against microtiter plates coated with formalinized bacterial cell material. Positive supernatants may be subjected to further screening to identify clones that secrete antibodies with the greatest affinity for the cariogenic organisms. In a preferred embodiment, clones with titers at least three times higher than background are screened again using an immunoprecipitation against denatured cell wall material from *S. mutans*. In a preferred embodiment, three clones were identified which bound detectably only to *S. mutans* strains ATCC25175, LM7, OMZ175 and ATCC31377. These clones were deposited with the American Type Culture Collection, receiving Deposit Numbers HB-1 2560, 12599 and 12558.

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2. Preparation of Monoclonal Antibodies Capable Of Eliciting An
Effector Response From Human Immune System

5 Previous efforts to develop an immunological method for the
prevention of dental caries employed heterologous antibodies. For example,
Lehner, United States patent 5,352,446, referred to use of monoclonal antibodies
to *S. mutans* surface antigens raised in mice in inhibiting the proliferation of those
bacteria in monkeys. More recently, Ma et al. *Nature Medicine*, 45(5) 601-6
(1998), reported similar results in humans, using a genetically engineered
10 secretory monoclonal antibody to *S. mutans* expressed in tobacco plants.
Drawbacks to this approach include 1) administration of heterologous antibodies
may aggregate the offending organisms, but will not kill them because such
antibodies will not elicit an immune response; and 2) repeated administration of
the antibody may elicit an immune response from the patient to the antibody. A
preferable approach is to use recombinant techniques to prepare chimeric
15 antibody molecules directed specifically to surface antigens of cariogenic
organisms, that will also elicit an effector response from the human immune
system (when used in man) upon binding to the target organism. This can be
accomplished by inserting variable regions or complementarity determining
20 regions ("CDR's") from mouse monoclonal antibodies that are specific to
cariogenic organisms into antibodies of the IgG and/or IgM classes from the
mammal to be treated. When the mammal to be treated is man, the antibodies
are said to be "humanized."

25 There are various ways to obtain nucleic acid sequences that
code on expression for human or humanized monoclonal antibodies to surface
antigens of cariogenic organisms: 1) Isolating mouse hybridomas which produce
monoclonal antibodies against cariogenic organisms and cloning mouse genes
that code on expression for those antibodies; 2) Using purified cariogenic
organisms to screen a phage display random library made from human B
30 lymphocytes to obtain genes that encode antibodies specific for cariogenic
organisms; 3) Isolating human hybridomas that produce monoclonal antibodies
against cariogenic organisms, using B lymphocytes recovered from heavily

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infected patients and cloning the human genes encoding for these antibodies; or
4) Immunizing human B lymphocytes and spleen cells *in vitro* using purified
cariogenic organisms, followed by fusion to form hybridomas to create immortal
cell lines. The techniques required are known to those skilled in the art.

In the presently preferred embodiment of the invention, mouse
monoclonal antibodies are "humanized." Using the PCR or Southern blot
technique, DNA fragments encoding the variable domains of mouse hybridomas
secreting antibody specific to cell surface antigens of cariogenic organisms are
isolated. Using gene cloning techniques, the variable regions of human IgG and
IgM immunoglobulin are replaced with the corresponding mouse variable regions
or CDR's. The result of this genetic engineering is a chimeric antibody molecule
with variable domains that selectively bind to surface antigens of cariogenic
organisms, but which interacts with the human immune system through its
constant regions to trigger a humoral immune response.

3. Administration of Monoclonal Antibodies

In order to prepare a sufficient quantity of monoclonal antibodies
for clinical use, the desired cell line transfected with IgG or IgM encoding
sequences must be propagated. Existing technology permits large scale
propagation of monoclonal antibodies in tissue culture. The transfected cell lines
will secrete monoclonal antibodies into the tissue culture medium. The secreted
monoclonal antibodies are recovered and purified by gel filtration and related
techniques of protein chemistry.

In experimental studies, monoclonal antibodies to *S. mutans* have
been applied directly to the surface of teeth. Application by ingestion of
mouthwash, or by chewing gum has also been proposed. A presently preferred
alternative is to express the monoclonal antibodies of the present invention in
edible plants, such as banana or broccoli. Eating plants transformed in
accordance with this invention will result in application of the antibodies to
cariogenic organisms present on tooth surfaces, and elsewhere in the mouth. It is
also contemplated that other organisms,

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both plant and animal may be transformed to express the monoclonal antibodies described herein, so that such antibodies may be ingested, for example, by drinking milk.

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Examples1. Producing mouse monoclonal antibodies against *S. mutans*

Type c *S. mutans* strain ATCC25175 are grown to log phase in BHI medium and washed twice with phosphate buffered saline, pH 7.2 (PBS), by centrifugation at 3000xg for 5 min. The pellet is resuspended in 1% formalin/0.9% NaCl, mixed at room temperature for 30 min and washed twice with 0.9% NaCl. BALB/c mice (8-10 weeks) are immunized intraperitoneally with 100 μ l of the antigen containing approximately 10^8 whole cells of formalinized intact *S. mutans* bacteria emulsified with Freund's incomplete adjuvant (FIA). After 3-5 weeks, mice will receive a second dose of antigen (10^8 whole cells of bacteria in FIA). Three days prior to fusion, the mice are boosted intravenously with 10^8 whole cells in saline.

The standard tissue culture media is RPMI 1640 (Gibco) medium supplemented with 2 mM L-glutamine, 1mM sodium pyruvate, and 10 mM HEPES and containing 100 μ g/ml penicillin and 100 / μ g/ml streptomycin with 10% fetal calf serum. Hybrids are selected in media containing HAT (100 μ g Hypoxanthine, 0.4. μ M Aminopterin; 16 μ M Thymidine). HT (100 μ g Hypoxanthine; 16 μ M Thymidine) is maintained in the culture medium for 2 weeks after aminopterin is withdrawn. OPI (1 mM oxaloacetate, 0.45 mM pyruvate and 0.2 U/ml bovine insulin) is added as an additional growth factor to the tissue culture during cloning of hybridomas. Hybridomas are raised according to the procedure reported by Liddell and Cryer (A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, Chichester, England, 1991). The NSI/A94.1 mouse myeloma cell line is used as the fusion partner and grown in spinner cultures in 5% CO₂ at 37°C and maintained in log phase of growth prior to fusion.

The following approach is used for screening for species-specific monoclonal antibodies against *S. mutans*. The initial screening is performed using an ELISA assay, which selects for the culture supernatants containing antibodies that bind to *S. mutans*. Formalinized bacteria are diluted in PBS to

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OD₆₀₀ = 0.5, and added to duplicate wells (100 μ l) in 96 well PVC ELISA plates preincubated for 4 h with 100 μ l of 0.02 mg/ml Poly-L-lysine. These antigen-coated plates are incubated overnight at 4°C in a moist box then washed 3 times with PBS and blocked with 0.5% fetal calf serum in PBS and stored at 4°C. 100 μ l of mature hybridoma supernatants are added to the appropriate wells of the antigen plates, incubated for 1 h at room temperature, washed 3 times with PBS-0.05% tween 20, and bound antibody is detected by the addition of polyvalent goat-anti-mouse IgG antibody conjugated with alkaline phosphatase diluted 1:1000 with PBS-1% fetal calf serum. After the addition of the substrate, 1 mg/ml p-nitrophenyl phosphate in carbonate buffer (15 mM Na₂CO₃, 35 mM NaH₂CO₃, 10 mM MgCl₂ pH 9.6), the color developments after 15 min is measured in an EIA reader at 405 nm. The positive supernatants (3 fold higher than control) are then subjected to the immunoprecipitation assay (mixing 100 μ l bacteria with 100 μ l supernatant) to screen for those with strong positive reactivity with *S. mutans*. The deposited clones were prepared according to this method.

2. Generating mouse/human chimeric genes which encode humanized monoclonal antibodies against *S. mutans*.

Described here is one of the ways to humanize mouse monoclonal antibodies. Genomic DNA of mouse hybridoma cell lines is isolated using the QIAamp system (Qiagen, Valencia, CA). After digestion with various restriction enzymes, DNA fragments are fractionated through 0.8% agarose gel by electrophoresis and transferred to a nitrocellulose membrane. Southern blotting is performed to identify the immunoglobulin gene. The heavy chain gene is probed with a DNA fragment from a mouse IgG heavy-chain gene that includes the J3 and J4 segments and the enhancer region. The light chain gene is probed with a DNA fragment from a mouse IgG light-chain gene containing J1-5 segments.

DNA restriction fragments of the selected size identified through Southern blot analysis are purified from agarose gel using Qiagen DNA Clean-up and Gel extraction system. The DNA is ligated into the Lambda-Zap 11 vector

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(Stratagene) to construct heavy- and light-chain libraries of these mouse hybridomas in lambda phage. The libraries are screened with heavy- and light-chain J-region probes as mentioned above. DNA of the positive clones is isolated, subcloned and sequenced. To achieve the best accuracy, both sense and antisense strands are sequenced. BLAST search is employed to translate the nucleotide sequence into the amino acid sequence and compare it with the existing antibody genes. The variable region of the heavy-chain is identified, subcloned and inserted into an expression vector which contains a DNA fragment encoding the human IgG heavy chain constant region and the *Ecogpt* gene providing resistance to mycophenolic acid. The variable region of the light-chain is also identified, subcloned and inserted into another expression vector which includes a DNA fragment encoding the human IgG light chain constant region and the neo gene giving resistance to G418.

3. Expressing Monoclonal Antibodies to *S. mutans* In Transformed Organisms

a) Producing human or humanized monoclonal antibodies in animal cells

The heavy and light chain of a human IgG gene are separately introduced or cotransfected into an animal cell line (such as SP2/0) using a lipofection reagent (BRL, Grand Island, NY). The transfected cells are incubated at 37°C in a 5% CO₂ atmosphere in 1x zinc option medium for 24 h and then in medium containing 10% fetal bovine serum. After 48 h incubation, the cells are transferred to a microtiter plate and grown in selection medium containing G418 and mycophenolic acid. The supernatants of drug-resistant cells are collected and screened for immuno-reactivity against *S. mutans* using the ELISA or precipitation assays mentioned above.

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b) Producing human or humanized monoclonal antibodies in edible plants

5 Transgenic plants have been recognized as very useful systems to produce large quantities of foreign proteins at very low cost. Expressing human or humanized monoclonal antibodies against *S. mutans* in edible plants (vegetables or fruits) allows direct application of plant or plant extracts to the mouth to treat existing dental caries and to prevent future bacterial infection. The
10 choice of transgenic, edible plants includes, but is not limited to, potato, tomato, broccoli, and banana.

Presented here are the procedures to produce transgenic Arabidopsis, an edible plant closely related to *Brassica* species including common vegetables such as cabbage, cauliflower and broccoli. It is chosen because many genetic and biochemical tools have been well developed for this
15 plant. There are several strategies to express IgG in this plant. One strategy is to first introduce the human IgG genes encoding the heavy chain and light chain to two separate transgenic lines. The two genes are brought together by genetic crossing and selection. Other methods involve sequential transformation, in which transgenic lines transformed with one IgG gene are re-transformed with the
20 second gene. Alternatively, genes encoding the heavy chain and light chain are cloned into two different cloning sites in the same T-DNA transformation vector under the control of two promoters, and the expression of both genes can be achieved by the transformation of a single construct to plant. Technically, the separate transformation method is the simplest one and it usually results in higher antibody yield. Therefore, we present this strategy here. It is possible to transform other plants using similar techniques.

25 The DNA fragments encoding the heavy and light chains of a human IgG gene are separately cloned into a Ti plasmid of *Agrobacterium tumefaciens*. The plasmid contains a promoter to express human heavy and light chains of IgG in *Arabidopsis thaliana*, an antibiotic marker for selection in *Agrobacterium tumefaciens* and an herbicide resistance gene for transformation selection in *Arabidopsis*. An *Agrobacterium tumefaciens* strain is transformed

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with these plasmids, grown to late log phase under antibiotic selection, and resuspended in infiltration medium described by Bethtold et al. (C.R. Acad. Sci. Paris Life Sci. 316:1194-1199, 1993).

5 Transformation of *Arabidopsis* by Ti-plasmid containing
Agrobacterium tumefaciens is performed through vacuum infiltration. Entire
plants of *Arabidopsis* are dipped into the bacterial suspension. The procedure is
performed in a vacuum chamber. Four cycles of 5 min vacuum (about 40 cm
10 mercury) are applied. After each application, the vacuum is released and
reapplied immediately. After infiltration, plants are kept horizontally for 24 h in a
growth chamber. Thereafter, the plants are grown to maturity and their seeds are
harvested. The harvested seeds are germinated under unselective growth
condition until the first pair of true leaves emerged. At this stage, plants are
sprayed with the herbicide Basta at concentration of 150 mg/l in water. The
15 *aribidopsis* plants containing transformed Ti plasmids are resistant to the
herbicide while the untransformed plants are bleached and killed. Such a
selection continues to the second generation of the plants. For the resistant
plants, total genomic DNA is isolated and probed with the DNA fragments
encoding heavy and light chains of the IgG gene. The plant extracts from the
20 positive transformants are prepared and screened for the expression of human
IgG protein with Western blot using antibodies against heavy and light chains of
constant regions of human IgG.

The plants expressing human IgG heavy chain are sexually
crossed with plants expressing human IgG light chain to produce progeny
expressing both chains. Western blotting is used to screen the both heavy and
light chains. Extracts from positive transformants are collected and screened for
25 immuno-reactivity against *S. mutans* using the ELISA or precipitation assays
mentioned above.

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4. Using human or humanized monoclonal antibodies against
S. mutans to treat or prevent human dental caries

With the successful completion of the above studies, humanized monoclonal antibodies against *S. mutans* are obtained. The plant tissue is tested for efficacy.

Plant tissue extracts containing monoclonal antibodies to *S. mutans* are mixed with various concentrations of *S. mutans* in the presence and absence of purified human complement components or purified human polymorphonuclear neutrophilic leukocytes. After a two hour incubation, the mixtures are plated onto BHI plates to examine the bactericidal activity.

Using the artificial plaque formation system developed by Wolinsky et al. (J. Dent. Res. 75:816-822, 1996), plant tissue extracts containing monoclonal antibodies are used to examine the ability of the expressed monoclonal antibodies ability to kill *S. mutans* in saliva or in existing dental plaques on artificial dental enamel. Analogous techniques are used to examine the ability to prevent the formation of dental plaques.

Human clinical trials are performed using these monoclonal antibodies produced through animal cells or plants. Human volunteers are treated with or without these human monoclonal antibodies against *S. mutans*. Then the level of *S. mutans* in saliva and in dental plaques is examined. The correlation between present and future dental caries in relationship with treatment of monoclonal antibodies is also examined.

It should be understood that the foregoing examples are for illustrative purposes only, and are not intended to limit the scope of applicants' invention which is set forth in the claims appearing below.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X | <p>SHARMA ARUN K ET AL: "Transgenic plants for the production of edible vaccines and antibodies for immunotherapy." CURRENT SCIENCE, vol. 77, no. 4, 25 August 1999 (1999-08-25), pages 524-529, XP001002438 Bangalore abstract page 524, right-hand column, paragraph 2 -page 525, left-hand column, paragraph 2 page 527, left-hand column, paragraph 3 -right-hand column, paragraph 1 page 528, left-hand column, paragraph 2 -right-hand column, paragraph 1 --- -/--</p> | 1-13,15 |



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X | <p>US 6 046 037 A (LEHNER THOMAS ET AL) 4 April 2000 (2000-04-04) column 1, line 13-17 column 4, line 59-63 column 5, line 22-24, 41-45 column 5, line 61 -column 6, line 11 column 6, line 63-67 column 19, line 55-62 column 20, line 5-31, 50-56 column 21, line 37-43 column 33, line 19-23, 31-34 examples 1-6 claims 1, 2, 20, 22</p> <p>---</p> | 1-13, 15 |
| X | <p>WO 00 11037 A (SHI WENYUAN ;HUME WYATT (US); UNIV CALIFORNIA (US)) 2 March 2000 (2000-03-02) page 1, line 16-21 page 6, line 1-16 page 9, line 10-12 page 14, line 18-21 page 15, line 5-9 page 16, line 29 -page 17, line 2 page 17, line 19-21 page 18, line 26 -page 19, line 4 claims 1-3</p> <p>---</p> | 1-3, 6-9, 12 |
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